

## SHORT COMMUNICATION

### HEAVY METALS IN CUTICULAR STRUCTURES OF PALPIGRADI, RICINULEI, AND SCHIZOMIDA (ARACHNIDA)

**Bruce Cutler:** Microscopy & Analytical Imaging Laboratory and Department of Ecology & Evolutionary Biology, University of Kansas, 1200 Sunnyside Avenue, Lawrence, Kansas 66045-7534 USA. E-mail: bcutler@ku.edu

**Lynn McCutchen:** Biology Department, Kilgore College, Kilgore, Texas 75662 USA

**ABSTRACT.** Samples from Palpigradi, Ricinulei, and Schizomida were examined by energy dispersive X-ray spectroscopy (EDS) for the presence of metallic elements in cuticular structures. Manganese was found in Ricinulei while zinc was found in Palpigradi and Schizomida. When presence or absence of zinc is superimposed on a cladogram of arachnid orders, its absence in the Acaromorpha (Acari + Ricinulei) appears to be a derived condition. Similarly the absence of manganese in the Uropygi (Schizomida + Telyphonida) may be synapomorphic as well.

**Keywords:** EDS, manganese, zinc

The presence of heavy metals in arthropod cuticular structures was first demonstrated by Hillerton et al. (1982) and Hillerton & Vincent (1982) in insects. How these metals are bound within the cuticle is still not known, although the specialized regions containing them are characterized by increased hardness (Hillerton et al. 1982; Schofield 2001). Heavy metals in arachnid cuticle was first investigated by Schofield & Levere (1989) and Schofield et al. (1989). In six spider species of four families (Araneidae, Lycosidae, Theraphosidae, Theridiidae), they found zinc in distal regions of fangs, and manganese in cheliceral teeth and tarsal claws. In two scorpion species from two families (Buthidae, Vaejovidae), they found manganese and zinc in cheliceral teeth in both species and iron in the buthid tissue as well. Schofield (2001) summarized results of examination of representatives of all arachnid orders except the Palpigradi, Ricinulei and Schizomida in an extensive table listing heavy metal occurrences in mechanical structures of animals. In addition to cheliceral structures he examined telsons and leg elements.

In the present paper we report on our examination of cuticular heavy metal elements of the three orders not included in the earlier studies:

**Palpigradi** (Prokoeneniidae)—*Prokoenenia wheeleri* (Rucker 1901), immature; USA: Texas: Travis Co., Lake Travis (30°25'N, 98°00'W), 24 July 2004.

**Ricinulei** (Ricinoididae)—*Pseudocellus* cf. *pelaezi* (Gutiérrez 1970), female; MEXICO: *Tamaulipas*: between kilo posts 4 and 5 off Highway 85

to Gomez Farias (23°02'N, 98°00'W), 16 May 2001.

**Schizomida** (Hubbardiidae)—*Stenochrus mexicanus* (Rowland 1971), female; MEXICO: *Tamaulipas*: between kilo posts 4 and 5 off Highway 85 to Gomez Farias (23°02'N, 98°00'W), 16 May 2001.

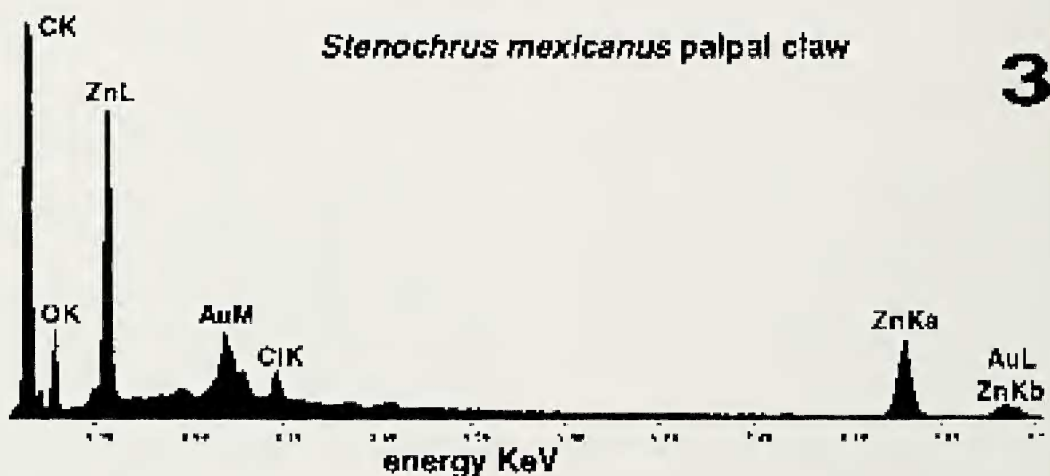
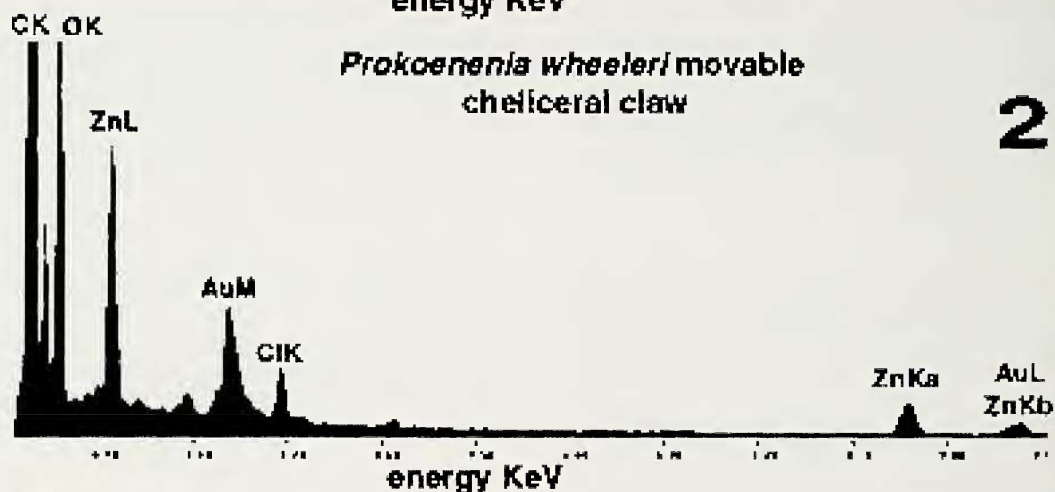
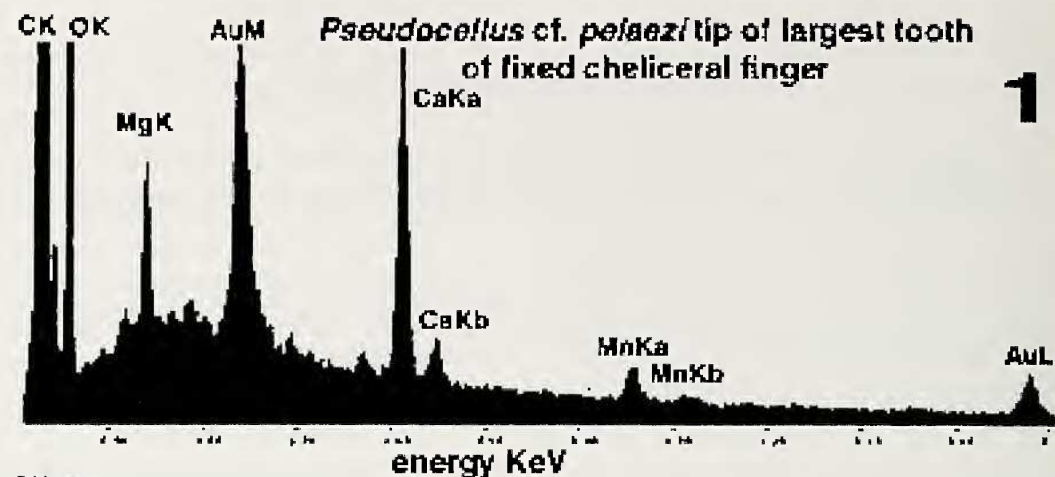
Structures analyzed were:

*Prokoenenia*—cheliceral body, movable finger, fixed finger, fourth tooth from distal end of fixed finger; leg 1 and 3 tarsal claws.

*Pseudocellus*—cucullus, cheliceral body, movable finger, fixed finger, largest tooth of fixed finger; palpal fixed finger, largest tooth of fixed finger; leg 1 and 4 tarsal claws.

*Stenochrus*—cheliceral body, movable finger, fixed finger; palpal tarsal claw, tarsal spur distal and proximal; leg 2 and 3 tarsal claws.

Specimens, collected by L. McCutchen and preserved in 70% ethanol, were dissected in fresh 80% ethanol and thus destroyed during processing. The parts were placed in 95% ethanol for 3 × 30 min, then 5 × 30 min in acetone, air dried out of acetone and mounted on SEM stubs with conductive carbon tabs and carbon paint. After being mounted, they were sputter coated with 10 nm of gold. Gold was chosen over carbon as a conductive coating, because of its superior electrical conductivity compared to carbon. The only problem encountered in relation to the elements of interest was the interference overlap of the gold La peak (9.71 keV) with the zinc K $\beta$  peak (9.57 keV). However, the zinc K $\alpha$



Figures 1-3.—Representative EDS spectra, non-background corrected, major elemental peaks are identified, the small peak between CK and OK is NK. Peaks are labeled by their elemental symbol and emitted X-ray type. 1. *Pseudocellus* tip of largest tooth of fixed cheliceral finger. MnKa peak (213 counts) at 5.90 KeV, MnKb peak (83 counts) at 6.49 KeV; 2. *Prokoenenia* tip of moveable cheliceral claw, ZnKa peak (617 counts) at 8.63 KeV, ZnLa peak (4960 counts) at 1.01 KeV; 3. *Stenochrus* tip of palpal claw, ZnKa peak (1077 counts) at 8.63 KeV, ZnLa peak (4213 counts) at 1.01 KeV.

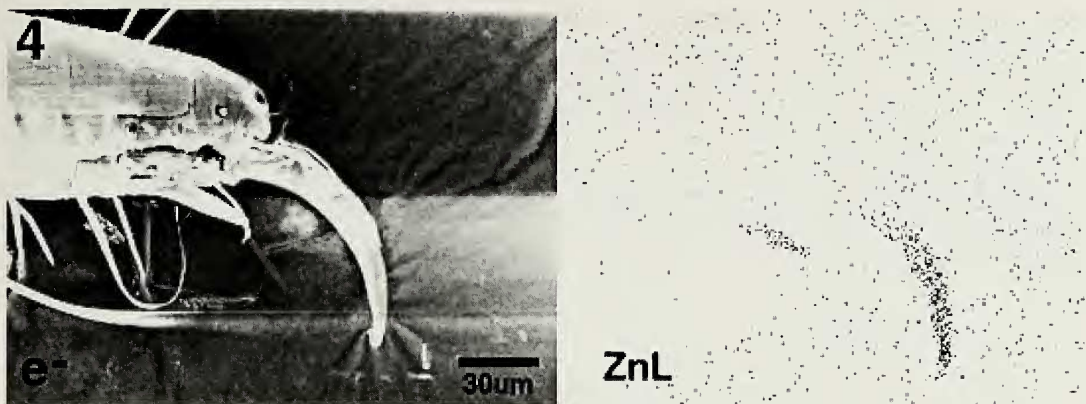


Figure 4.—Mapping of ZnL x-rays in the distal tarsal region of the palpus of *Stenochrus*. e<sup>-</sup> is the secondary electron image (conventional SEM image, left) scanned at the same rate as the ZnL family x-ray map (ZnL, right). Note increased density of ZnL family pixels in the distal part of the tarsal spur and the claw. Original dimensions = 1024 × 800 pixels.

peak can be confirmed by the high zinc La peak (1.01 keV) in the same spectra.

Specimens were analyzed in a LEO 1550 field emission scanning electron microscope (SEM); equipped with an EDAX energy dispersive x-ray system (EDS), with an ultra-thin window Phoenix detector using Genesis software. All spectra were collected by focusing the electron beam at the tip of the structure except for the cheliceral body spectra which were collected from the center of the dorsal side in *Prokoeninia* and *Stenochrus*, and the center of the ventral side in *Pseudocellus*. Excitation voltage was 20 KeV and the working distance was 8 mm. Collecting times were 300–400 counting seconds. Beam current was measured using a Faraday cup. For the standard aperture used (30 µm) current was 245 pA, for the *Pseudocellus* cucullus a 60 µm aperture was used, current was 1.06 nA; for the *Pseudocellus* cheliceral body a 20 µm

aperture was used, current was 111 pA. Major X-ray peaks were considered as positive when they had a minimum detectable level (MDL) of 99.9% certainty, MDL > background + 3 (square root of background) (Lee 1993).

Zinc was detected in leg and palpal claws and distal cheliceral areas of *P. wheeleri* and *S. mexicanus*. Manganese was detected in one of the cheliceral teeth of *P. cf. pelaezi*. Calcium peaks are often associated with manganese peaks in arachnids as seen in Fig. 1. In *P. cf. pelaezi* a magnesium peak often appeared with the calcium peaks whether associated with manganese or not. Representative spectra are shown in Figs. 1–3 while Fig. 4 shows an x-ray map of the distal palpal tarsus in *S. mexicanus*.

Energy dispersive x-ray spectroscopy (EDS) results in biological materials are considered to be qualitative because of the uncertain composition and heterogeneity of the materials being examined. Schofield and collaborators utilized particle induced x-ray emission (PIXE) and scanning transmission ion microscopy (STIM), which allow deep or complete penetration of the specimen. Using tomography based on these methods, one can achieve quantitative elemental results as well as deep element mapping. The disadvantage of using these methods is the limited availability of the instrumentation. Despite its qualitative nature in biology, EDS has the advantage of being far more available since many SEMs have EDS detectors installed for use in the geological and materials sciences. Qualitative results in EDS are readily attained in a short time frame making it an ideal surveying tool.

This study adds cuticular metallic element data for the three arachnid orders not included in Schofield's (2001) summary. When the distribution of the elements is superimposed on the cladogram of



Figure 5.—Phylogeny of Arachnida based on Shultz (1990) with presence of cuticular metallic elements added. Palpigradi, Ricinulei, Schizomida from this paper, all others from Schofield (2001).



Shultz (1990) (Fig. 5), the near ubiquity of zinc is obvious. Absence of zinc in the cuticle of Acaromorpha (Acari and Ricinulei) may be a synapomorphy of these orders. Similarly the absence of manganese in the Uropygi (Schizomida and Thelyphonida) may be synapomorphic as well. Absence of cuticular zinc may be a derived condition in Opiliones. However, both the Acari (two species in Schofield (2001), one by B. Cutler unpublished) and the Opiliones (three species in Schofield (2001), two by B. Cutler unpublished) have been very poorly sampled relative to the size of the orders. These orders should be investigated more thoroughly to check for the presence of zinc. Likewise, the lack of iron in the Solifugae (two species investigated, Schofield (2001)) indicates that a wider range of taxa within this order should be examined given its close relationship to Scorpionida and Pseudoscorpiones.

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